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COLLISELLA AUSTRODIGITALIS SP. NOV.: A SIBLING SPECIES OF LIMPET (ACMAEIDAE) DISCOVERED BY ELECTROPHORESIS

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Mayr (1963, p. 34) defines sibling species as "morphologically similar or identical natural populations that are reproductively isolated." During the past decade biochemical analyses have confirmed the differences and genetic isolation of numerous siblings that were originally recognized on the basis of breeding tests, cytological comparisons, or slight morphological differences. Included in such studies have been species of *Drosophila* (Hubby and Throckmorton, 1968; Prakash, 1969; Ayala, Mourão, Pérez-Salas, Richmond, and Dobzhansky, 1970), mammals (Johnson, Selander, Smith, and Kim, 1972), and seastars (Schopf and Murphy, 1973). In one instance biochemical data provided the first evidence that what had been considered a single species of sea cucumber was actually two morphologically similar species (Manwell and Baker, 1962; Manwell, 1966), and in the marine polychaete worm *Capitella capitata* (Fabricius, 1780) six sibling species have been distinguished on the basis of electrophoretic patterns of enzyme loci (Grassle and Grassle, 1976).

Limpets currently classified as *Collisella digitalis* (Rathke, 1833) on the basis of morphology range from the Aleutian Islands to southern Baja California (McLean, 1969). They are common throughout this range in the middle and upper intertidal zones, and are among the most eurytopic and phenotypically plastic of the eastern Pacific Acmaeidae. In the course of a geographic survey of two enzyme coding loci, two partially sympatric, reproductively isolated units were discovered within this taxon.

Materials and Methods

Locations

Ten localities, covering a range of 1200 air kilometers, were sampled (Fig. 1). In July and August of 1977 samples were collected from the following sites in California: Corona del Mar State Beach (COR); Point Mugu (MUG); Gaviota State Beach (GAV); Jalama County Park, Santa Barbara County (JAL); Cayucos State Beach (CAY); the mouth of Mill Creek, 7 km south of Lucia (MIL); the Hopkins Marine Station, Pacific Grove (PAC); the mouth of Younger Lagoon, Santa Cruz (SAN); and the west side of Bodega Head, Sonoma County (BOD). Yachats State Park, Yachats, Oregon (YAC) was sampled in March, 1977.

Method of collection

Collisella digitalis exists in at least two ecotypic forms. One of these is found in beds of the stalked barnacle, *Pollicipes polymerus* (Sowerby, 1833), and the

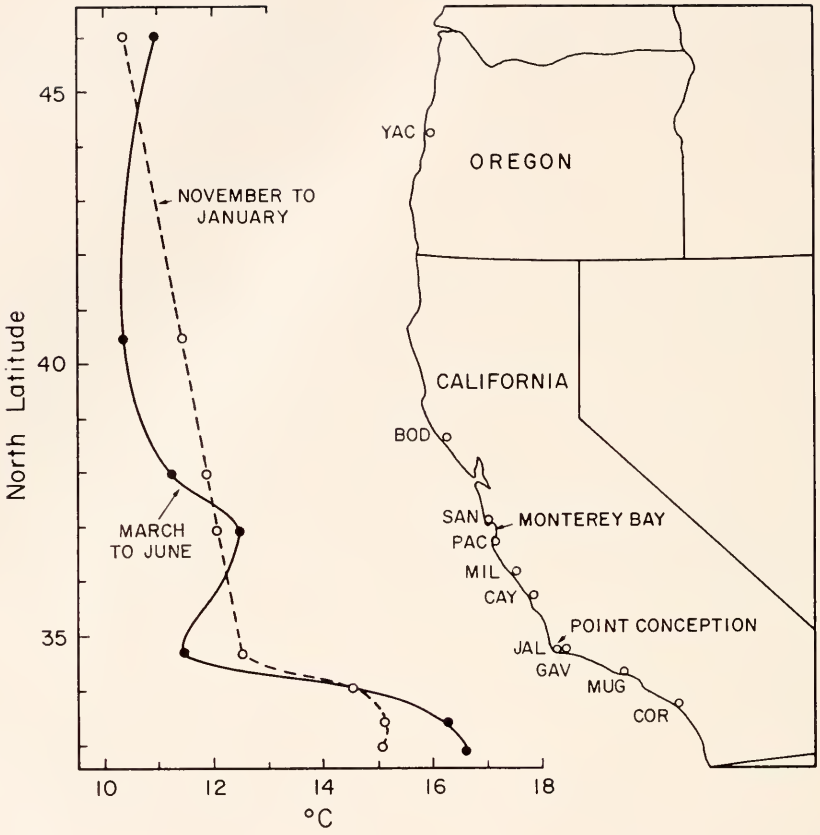

FIGURE 1. Map of California and Oregon showing locations of sampling sites. Graph of mean March to June and November to January surface temperatures shows a thermal break at Point Conception and anomalous conditions near Monterey Bay (after Sverdrup, Johnson, and Fleming, 1942, p. 724).

other is found higher on intertidal rock surfaces. Giesel (1970) has suggested that phenotypic plasticity alone does not account for these morphs, but that predation pressure may select them from a continuum of shell types during each post-larval generation. Therefore, to assure as representative a sample of genotypes as possible, approximately equal numbers of specimens were collected at each site from stalked barnacle beds and from higher zones on the same rocks, except at YAC where the barnacle beds were not sampled.

Because it is difficult to identify small limpets to species, only specimens longer than 5 mm were collected. Otherwise, no conscious selection of any size class or shell pattern was made.

Limpets were brought alive to the laboratory and then kept at −76° C until electrophoretic analysis.

Electrophoresis

The digestive glands of individual snails were removed and homogenized in 1 ml polethylene BEEM capsules using the roughened ends of glass rods as pestles. The capsules were centrifuged at $4500 \times g$ for 10 min and the supernatant absorbed onto 5×6 mm pieces of Whatman 3MM filter paper for insertion into starch gels. Gel composition, electrophoresis, and the leucine aminopeptidase (Lap) staining method have been described (Murphy, 1976). Phosphoglucose isomerase (Pgi) alloenzymes were visualized by staining for 5 to 10 min in a solution of 2 mg phenazine methosulfate, 7 mg NADP, 15 mg MTT, 20 mg fructose-6-phosphate, 30 units of glucose-6-phosphate dehydrogenase, and 160 mg $MgCl_2 \cdot 6H_2O$ in 70 ml 0.05 M Tris-HCl buffer, pH 8.0. Horse ferritin (Sigma) was used as a marker, and individuals of known genotypes served as standardizations of mobility variants between samples.

Results

Lap polymorphisms

The zymograms reveal one major zone of Lap activity, and, assuming a correspondence between bands and alleles, a total of 10 codominant alleles were found at the locus coding for this zone. The Lap phenotypes of homozygotes are double bands about 4 mm apart, and those of heterozygotes are four bands, or three where the fast band of the slower allele has the same mobility as the slow band of the faster allele (Fig. 2). Allele abbreviations follow Ayala, Powell, Tracey, Mourão, and Pérez-Salas (1972). The most common allele

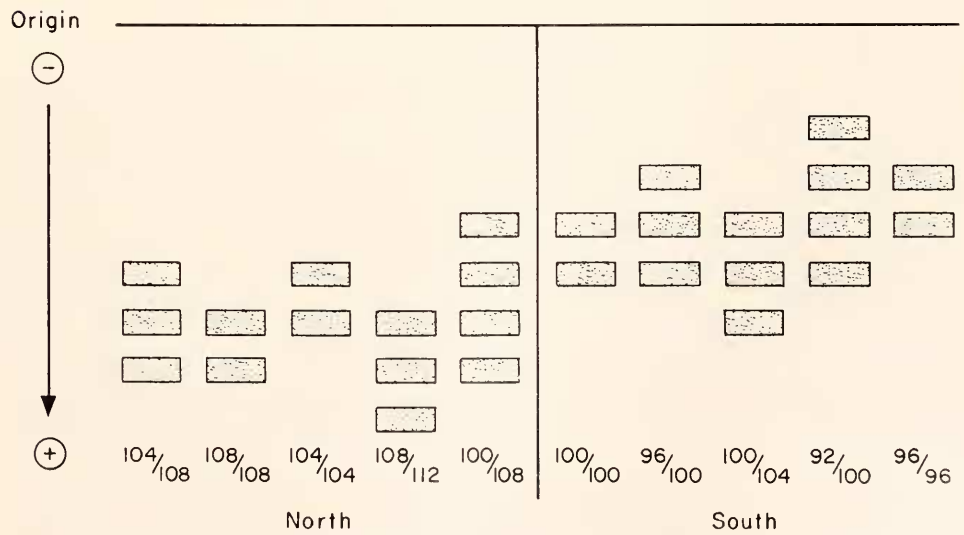

FIGURE 2. Banding patterns of the five most frequent Lap phenotypes in the "north" and "south" groups.

TABLE I

Observed genotypes, allele frequencies, and chi-square goodness of fit tests at the Lap locus.

	Localities									
	YAC	BOD	SAN	PAC	MIL	CAY	JAL	GAV	MUG	COR
Genotypes										
88/100	0	0	0	0	0	1	0	0	1	1
92/92	0	0	0	0	0	0	0	1	1	1
92/96	0	0	0	1	0	0	1	3	0	5
92/104	0	0	0	1	0	0	0	0	2	1
94/100	0	0	0	0	0	0	1	1	0	1
96/96	0	0	0	1	0	2	5	1	2	4
96/102	0	0	0	0	0	0	0	0	1	1
96/104	2	0	0	1	0	1	4	1	3	1
98/100	0	0	0	0	0	0	4	1	2	0
92/100	0	0	0	4	2	8	8	8	9	11
96/100	0	0	0	4	3	14	13	22	27	22
100/100	0	1	0	43	8	52	59	58	79	106
100/104	0	0	1	4	4	5	4	6	10	13
100/102	0	0	0	0	0	0	0	0	0	1
104/104	9	31	23	13	28	14	14	3	1	0
108/108	22	45	47	11	49	12	12	0	0	0
104/108	20	53	50	20	57	29	18	0	0	0
100/108	0	1	3	1	1	0	1	0	1	1
96/108	1	0	0	0	4	3	1	1	0	0
102/108	1	0	0	0	0	0	0	0	0	0
100/112	1	0	1	0	0	0	0	0	0	0
104/112	2	0	1	0	1	0	0	0	0	0
108/112	1	5	1	0	1	0	0	1	0	0
112/112	1	0	0	0	0	0	0	0	0	0
Accord with Hardy-Weinberg										
X^2	1.47	3.85	2.12	86.1	18.1	76.9	83.1	1.61	0.671	2.79
df	2	2	2	4	4	6	5	3	3	3
P	0.48	0.15	0.35	<0.001	<0.01	<0.001	<0.001	0.66	0.88	0.43

in the five southernmost samples is used as standard, Lap 100. The genetic hypothesis is that the double bands represent two polymeric enzymes that share a common polymorphic subunit as well as having different monomorphic subunits (Shaw, 1964).

Genotype frequencies for this Lap locus were determined for all ten localities, and their agreement with Hardy-Weinberg (H-W) expected frequency distributions was tested. Because of the large number of alleles at this locus, many genotypic classes contain few or no individuals, and classes with expected frequencies fewer than 5 were not included in chi-square goodness of fit tests. From Table I it is clear that the Lap genotype frequencies from localities between PAC and JAL inclusive do not conform to H-W expectations. It is also evident that, while they do not deviate significantly from H-W expectations, samples from sites north

TABLE II

Chi-square accord of Lap genotype frequencies after subdivision of samples PAC, MIL, CAY, JAL, and GAV into "north" and "south" subsamples.

	PAC		MIL		CAY		JAL		GAV	
	n	s	n	s	n	s	n	s	n	s
N	45	59	141	17	57	84	46	98	5	102
X^2	0.178	1.43	2.28	i*	0.157	0.621	1.48	4.02	i	0.500
df	2	3	2	—	2	2	2	3	—	3
P	0.92	0.70	0.32	—	0.92	0.67	0.48	0.26	—	0.92

* i = insufficient sample.

of PAC and samples from south of GAV have very few genotypes in common. There are four alleles in the MUG-COR samples and one in the YAC-BOD-SAN samples that are not shared by both groups. To test the hypothesis that two reproductively isolated groups had been sampled, the alleles of the YAC, BOD, and SAN samples were pooled into a "north" sample and those of MUG and COR into a "south" sample, and the probability of correctly assigning an individual to one of the samples thus formed was calcualted by the method of Ayala and Powell (1972). This probability is 98.8% which is very close to the 98.9% probability based on the overlap of the observed genotypes, and matches, to the nearest per cent, the arbitrary value of 99% defined as diagnostic by Ayala and Powell. By this method those Lap genotypes listed below 100/102 in Table I have a higher expectancy in the "north" group, and those listed above 104/104 a higher expectancy in the "south" group. If individuals in the samples between PAC and GAV inclusive are each assigned to a "north" or "south" subsample on on this basis, the significant ($P < 0.05$) deviations from H-W equilibrium are eliminated in every case, and at GAV, where less than 5% of the genotypes fall into the "north" subsample, the "south" subsample has a better fit to H-W expectations than does the undivided sample (Table II).

These results indicate two genetically isolated groups, each with some endemic alleles, that have very different frequencies for the Lap alleles that they share. North of PAC and south of GAV these groups are allopatric, or virtually allopatric, and between PAC and GAV inclusive they are sympatric. Assuming no hybridization in the zone of sympatry, the expected numbers of the Lap genotypes 96/104, 96/108, 100/104, and 100/108 were calculated for each of the sympatric samples. The totals for each of these genotypes are 3.6, 3.9, 24, and 1.6, respectively, while the observed numbers of these genotypes are 7, 9, 23, and 3. Since these four genotypes would account for the vast majority of "north" × "south" hybrids, there is little, if any, introgression evident in the 654 individuals making up the five sympatric samples.

Pgi polymorphism

The results of the Lap analyses are corroborated by the survey of the one major zone of Pgi activity that could be consistently scored. The locus coding for

TABLE III

Observed Pgi genotypes and chi-square accord with Hardy-Weinberg expectations for samples after subdivision into "north" and "south" subsamples on the basis of Lap genotypes. Parentheses enclose statistic for samples prior to subdivision.

	Localities													
	YAC	BOD	SAN	nPAC	sPAC	nMIL	sMIL	nCAV	sCAV	nJAL	sJAL	nGAV	sGAV	MUG	COR
Genotypes															
88/88	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0
88/94	4	5	4	1	0	3	0	1	0	1	0	0	1	1	2
92/94	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
94/94	6	23	18	2	5	11	1	8	5	7	5	0	3	8	15
94/100	21	49	39	18	22	52	4	20	24	15	22	2	38	44	54
94/106	2	5	5	3	3	7	1	2	3	1	3	0	5	6	10
94/108	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
85/100	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0
88/100	4	1	6	4	0	10	0	3	1	1	3	0	0	4	1
92/100	0	0	1	0	0	0	0	0	0	0	0	0	1	1	0
100/100	20	42	38	14	24	45	8	22	39	12	38	3	43	62	75
100/106	3	10	13	2	5	12	2	0	10	2	1	0	9	9	11
100/108	0	1	0	0	0	0	0	0	0	0	1	0	0	1	1
100/112	0	0	1	0	0	1	1	1	0	0	1	0	0	1	0
88/106	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
106/106	0	0	1	0	0	0	0	0	1	0	1	0	1	1	0
Hardy-Weinberg accord															
X^2	0.103	2.15	3.07	0.657 | 0.014 (0.763)*		1.88 | 0.218 (1.46)		0.868 | 0.105 (1.57)		0.357 | 0.274 (2.84)		i | 2.62 (2.88)		0.502	4.69
df	2	4	5	1 | 5 (3)		5 | 1 (5)		2 | 2 (3)		2 | 1 (3)		— | 3 (3)		3	4
P	0.95	0.71	0.69	0.42 | >0.99 (0.86)		0.87 | 0.64 (0.92)		0.65 | 0.95 (0.67)		0.84 | 0.60 (0.42)		— | 0.45 (0.41)		0.92	0.32

this zone has a total of eight alleles, Pgi^{86} to Pgi^{112}, the allele used as standard being the most common in every sample. At this locus the electrophoretic phenotypes of homozygotes are single bands and those of heterozygotes are three bands, indicating a dimeric enzyme with a single polymorphic subunit.

The "north" and "south" groups established above on the basis of Lap genotypes differ much less with respect to the Pgi loci than they do for their Lap loci. None of the ten original samples differed significantly from H-W equilibrium, and division of the sympatric samples into "north" and "south" components results in only slightly better overall H-W agreement where Pgi genotypes are concerned (Table III). However, the mean frequencies of three of the four most common alleles, Pgi^{88}, Pgi^{94}, and Pgi^{100}, in the "north" and "south" sympatric subsamples are statistically different ($P < 0.05$ by Student's t-test). The mean frequencies of these alleles in the sympatric subsamples are clearly similar to their frequencies in the corresponding northern and southern allopatric samples (Table IV). Only a linkage disequilibrium of unprecedented scale could account for this, were not two genetically isolated groups involved.

Status of the "north" and "south" groups

Acmaeid limpets have a planktonic larval stage lasting at least five days (Kessel, 1964; Proctor, 1968) and probably as long as three weeks or more if a suitable settling site is not available (Karp, 1970). Such species should have homogeneous gene pools with similar allelic frequencies over long distances, if not over an entire species range (Gooch, 1975; Soulé, 1976). Therefore the presence, in a single species, of a cline of Lap allele frequencies of the magnitude found here between the northern and southern ends of the range sampled would not be expected.

The larger than expected numbers of Lap genotypes 96/104 at JAL, 96/108 at MIL and CAY, and 100/108 at SAN may represent hybrids between the "north"

TABLE IV

Comparison of Pgi 88, 94, 100, and 106 frequencies for "north" (n) and "south" (s) samples. Parentheses enclose statistics comparing means of n and s subsamples from PAC, MIL, CAY, JAL, and GAV.

	88		94		100		106	
	n	s	n	s	n	s	n	s
PAC	0.078	0	0.289	0.297	0.578	0.636	0.056	0.068
MIL	0.046	0	0.291	0.206	0.592	0.676	0.067	0.088
CAY	0.036	0.012	0.342	0.220	0.596	0.673	0.018	0.095
JAL	0.050	0.020	0.388	0.230	0.525	0.691	0.038	0.039
GAV	i	0.005	i	0.250	i	0.657	i	0.078
$\bar{X}$	0.053	0.0074	0.328	0.241	0.573	0.667	0.043	0.074
t	(5.00)		(3.17)		(5.25)		(1.98)	
P	(<0.01)		(<0.02)		(<0.01)		(<0.05)	
Mean of YAC, BOD, and SAN	0.043		0.347		0.546		0.059	
Mean of MUG and COR		0.014		0.265		0.652		0.062

and "south" groups. However, with respect to taxonomy, the existence of hybrids is not crucial. Dobzhansky, Ayala, Stebbins, and Valentine (1977, p. 171) define *species* as "Mendelian populations, or arrays of Mendelian populations, between which the gene exchange is limited or prevented by reproductive isolating mechanisms." The small number of possible hybrids and the differences in allele frequencies at the loci studied, despite the groups' approximately 350 km zone of sympatry, clearly indicate a sufficient degree of reproductive isolation to qualify these groups for species status.

All samples, even after subdivision into "north" and "south" subsamples, have deviations from H-W equilibrium that, although they are not statistically significant at the sample sizes employed, reflect an excess of homozygotes. Several other authors have noted similar H-W deviations in marine invertebrates, including several species of mussels (Koehn and Mitton, 1972; Koehn, Turano, and Mitton, 1973; Tracey, Bellet, and Gravem, 1975; Koehn, Milkman, and Mitton, 1976), a lobster (Tracey, Nelson, Hedgecock, Shleser, and Pressick, 1975), a phoronid (Ayala, Valentine, Barr, and Zumwalt, 1974), and a population of *Collisella digitalis* from Bodega Head (Gresham and Tracey, 1975). This is best explained by strong microhabitat selection, proportionally greater within than between microhabit mating, and subsequent mixing of the resulting larvae (see Mitton and Koehn, 1973; Tracey, Bellet, and Gravem, 1975; Tracey, Nelson, Hedgecock, Shleser, and Pressick, 1975; Koehn *et al.*, 1976 for similar explanations). High fecundity organisms such as these should easily afford the cost of such selection pressure (Williams, 1975). However, Makela and Richardson (1977), ignoring the probable selection pressure stemming from competition, argue that the presence of two or more sibling species is an equally good explanation of excess homozygosity that involves no selective cost. To account for the residual homozygote excess encountered here, their explanation requires that both the "north" and "south" groups be composed of more than one species with similar allelic frequencies and identical range end-points. The assumptions required for this hypothesis seem to be too stringent to warrant its consideration.

Taxonomy

The type locality for *Collisella digitalis* is Sitka, Alaska (Rathke, 1833). Therefore, the "south" group differentiated here is the unnamed species. I propose it be named *Collisella austrodigitalis* Murphy, sp. nov. A holotype and paratypes, all from Corona del Mar, California (33°35′ N, 117°52′ W) and voucher specimens of *Collisella austrodigitalis* and *C. digitalis* from sites where the species are sympatric, have been deposited with the California Academy of Sciences, San Francisco. Paratypes have been deposited with the United States National Museum of Natural History, Washington, D. C.

The holotype was collected from the high intertidal rock surface. A core of its tissue was removed with a hollow needle and its genotype, for the two loci studied, was determined to be homozygous Lap ^{100} and homozygous Pgi ^{100}.

Morphological differences

The morphological similarity of these species is attested to by the fact that "*Collisella digitalis*" has been a frequent subject of taxonomic and ecological studies, yet no one has suggested that populations from northern and southern California belong to different taxa. Eurytopic acmaeids such as these exhibit striking shell variation in response to different ecological conditions (Test, 1945, 1946) that tends to mask differences between similar species. After determining species electrophoretically, examination of shells from sites where these species co-occur reveals some subtle interspecific differences. The shell interior tends to be more darkly shaded and the interior margin less often tesselated in *Collisella austrodigitalis*, while the shell exterior of *C. digitalis* has a more mottled pattern. Due to the variation mentioned above, there is much interspecific overlap with respect to these characters. Shell sculpture appears to be a more dependable diagnostic feature. *C. digitalis* tends to have broad, rounded ribs with convex intercostals, while the tendency of *C. austrodigitalis* is to ribs with square cross sections and flat intercostals (see Fig. 3). The spines on the ribs of *C. austrodigitalis* give them a beaded appearance. However, the sculpture on either species may be obscured by erosion or other phenotypic responses to the environment.

The radulae of these species are very similar. McLean (1966) studied "*C. digitalis*" radulae from both north of San Francisco and south of Point Conception without noting important differences. Fritchman (1960) found that collections of "*C. digitalis*" from north and south of Point Conception differed significantly with respect to two of the six radular characters he studied. The diagnostic value of his characters would not be great, however, since large numbers of northern and southern individuals shared the same character states.

Distribution

Temperature is generally considered to be one of the primary factors controlling the distribution of marine invertebrates (Dunbar, 1963; Kinne, 1970). Point Conception is the approximate southern range end-point of *Collisella digitalis*, and Monterey Bay the approximate northern limit of *C. austrodigitalis*. Both of these geographic features are well known for their peculiar hydrographic regimes and biogeographic significance. Point Conception is the recognized boundary between the warm temperate, Californian, and temperate, Oregonian, molluscan provinces. It is the range end-point for several hundred species of either province (Valentine, 1966). The dramatic thermal break at Point Conception (Fig. 1) is presumed to account for its biogeographic importance. The sites JAL and GAV are only 30 km apart, one on either side of Point Conception, and GAV is less than 2 km farther south than JAL, yet, there is a striking difference in numbers of *Collisella digitalis* at these sites (Table I). The comparative reduction of this more northern of the two species at GAV correlates with the abrupt thermal change. Monterey Bay is generally warmer than waters to both the north and south, and harbors many southern thermophiles that end their ranges there (Valentine, 1966). The greater frequency of *C. austrodigitalis* at PAC than at MIL, even though PAC is farther north, may be a response to the

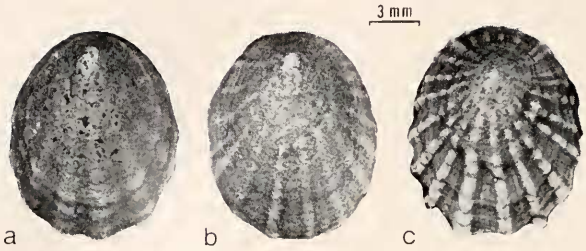

FIGURE 3. Shells of (a) *Collisella digitalis* from MIL; (b) *C. austrodigitalis* from MIL; (c) *C. austrodigitalis* from COR with sculpture similar to that of the holotype. Shells have been coated to accentuate sculpture.

higher temperatures near the bay. Addicott (1966) presents the possibility that unusually warm episodes of the annual Davidson Current period off central California (Bolin and Abbott, 1963) may result in blooms of southern species with planktonic larvae in this area. Recent hydrographic data (Lasley, 1977) indicate that such a warming cycle is in progress. Therefore, numbers of *C. austrodigitalis* in the vicinity of Monterey Bay may, at present, be higher than usual. Allelic frequencies previously reported for *C. digitalis* (Murphy, 1976) are in error because specimens of *C. austrodigitalis* were unwittingly included in the samples from the vicinity of Monterey Bay.

DISCUSSION

Allopatric speciation seems to be the only possible mode of origin that can explain siblings such as *Collisella austrodigitalis* and *C. digitalis* that are restricted to the same habitats in the intertidal zone. However, the barriers that might have served to divide spatially the original species are not obvious. One possible chain of events, involving any of the late Cenozoic world wide climatic fluctuations is as follows. During warming trends warm-water species extend their ranges into higher latitudes, and during cooling intervals, as the polar-equatorial temperature gradient increases, they contract their ranges equatorward (Valentine, 1973). During episodes of range contraction populations might become isolated in suitably warm, higher latitude embayments (Addicott, 1966 considered this as a possible explanation for the presence of a warm-water, late Pleistocene fossil assemblage in northern California). Should this isolation last long enough, speciation would occur. Golikov (1973) predicts that cold adaptation in such an isolated population would accelerate speciation. Then during a subsequent warming interval sympatry between the new, but still morphologically and ecologically similar, species might be reestablished. If this chain of events does explain the evolution of *Collisella austrodigitalis* and *C. digitalis*, then the southern *C. austrodigitalis* is the older species, and the isolation and cold adaptation, if not speciation, of *C. digitalis* was complete by the late Pleistocene since "*C. digitalis*" is present in a cool temperate, Aleutian, fossil assemblage of that date near Bandon, Oregon (Zullo, 1969). Investigation of other molluscan species with ranges overlapping two or more molluscan provinces may reveal other examples of such siblings.

Speculation as to the isolating mechanisms that prevent introgression of these siblings is difficult because of the paucity of information on acmaeid reproduction. Dissynchronization of spawning is a possibility, but Fritchman (1961), while observing some seasonality of spawning in *C. digitalis* in the vicinity of San Francisco, found that in favorable habitats some spawning occurred year around. Thus, it seems unlikely that this mechanism could account for the degree of isolation observed unless there is increased periodicity of spawning near the range end, perhaps due to brief annual exposure to critical temperatures there. Of the other possible mechanisms that could operate, gametic isolation seems unlikely to have evolved except in response to zygotic mortality or hybrid inviability, but either of these latter mechanisms is a sufficient, and perhaps most reasonable, explanation for the lack of introgression observed.

Whether or not introgression is entirely absent cannot be determined from the present data. Even *C. digitalis* from YAC and *C. austrodigitalis* from COR, the terminal sites of the range studied, have some Lap alleles in common, and, therefore, Lap genotypes that are identical to those that should be most frequent among hybrids. With the criterion used for distinguishing the species, and assuming no hybridization, the Lap genotypes 96/104 and 96/108, although rare, are approximately twice as frequent in the zone of sympatry as expected. This fact in conjunction with the three 100/108 and the one 100/112 Lap genotypes at SAN, which is well within the dispersal distance of the zone of sympatry, suggests a small amount of hybridization. However, a definitive study of this point will have to employ larger samples or additional loci. By including more loci, especially if other diagnostic loci can be found, not only can the frequency of hybrids be determined, but, if hybrids do exist, the extent to which F 1 hybrids backcross to either parental species may be estimated using the logic Hall and Selander (1973) applied to their study of hybridizing lizards.

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Summary

A geographic survey of protein polymorphism in the intertidal limpet *Collisella digitalis* reveals that populations from northern California and Oregon have allelic frequencies at a leucine aminopeptidase locus that are sufficiently different from populations in southern California that individuals can be correctly assigned to their geographic entity with a 98.8% probability on the basis of their genotype. These two groups also have different allelic frequencies at a phosphoglucose isomerase locus, but only slight morphological differences between the groups are evident.

Genotype frequency analysis shows that on the central California coast between Monterey Bay and Point Conception both the northern and southern groups are

present, but not interbreeding. Thus, these groups are sibling species. The southern species is herein named *Collisella austrodigitalis* sp. nov.

These species are thought to have originated by allopatric speciation, perhaps following the spatial isolation of a population of *C. austrodigitalis* during an equatorward contraction of that species' range in response to one of the late Cenozoic world wide cooling trends.

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